

B3

-- Soluble RANKL comprises a signal peptide and the extracellular domain or a fragment thereof. An exemplary signal peptide is that shown in SEQ ID NO:9; other signal (or leader) peptides are well-known in the art, and include that of murine Interleukin-7 or human growth hormone. RANKL is similar to other members of the TNF family in having a region of amino acids between the transmembrane domain and the receptor binding region that does not appear to be required for biological activity; this is referred to as a 'spacer' region. Amino acid sequence alignment indicates that the receptor binding region is from about amino acid 162 of human RANKL to about amino acid 317 (corresponding to amino acid 139 through 294 of murine RANKL, SEQ ID NO:11), beginning with an Ala residue that is conserved among many members of the family (amino acid 162 of SEQ ID NO:13). --

At page 5, please delete in its entirety the paragraph at lines 20-34, which begins with "Moreover, fragments of the extracellular domain..." and substitute therefor the following paragraph:

B4

-- Moreover, fragments of the extracellular domain will also provide soluble forms of RANKL. Those skilled in the art will recognize that the actual receptor binding region may be different than that predicted by computer analysis. Thus, the N-terminal amino acid of a soluble RANKL is expected to be within about five amino acids on either side of the conserved Ala residue. Alternatively, all or a portion of the spacer region may be included at the N-terminus of a soluble RANKL, as may be all or a portion of the transmembrane and/or intracellular domains, provided that the resulting soluble RANKL is not membrane-associated. Accordingly, a soluble RANKL will have an N-terminal amino acid selected from the group consisting of amino acids 1 through 162 of SEQ ID NO:13 (1 through 139 of SEQ ID NO:11). Preferably, the amino terminal amino acid is between amino acids 69 and 162 of SEQ ID NO:13 (human RANKL; amino acids 48 and 139 of SEQ ID NO:11). Similarly, the carboxy terminal amino acid can be between amino acid 313 and 317 of SEQ ID NO:13 (human RANKL; corresponding to amino acids 290 through 294 of SEQ ID NO:11). Those skilled in the art can prepare these and additional soluble forms through routine experimentation. --

At pages 25-26, please delete in its entirety the paragraph at page 25, line 29 to page 26, line 3, which begins with "A clone encoding a protein..." and substitute therefor the following paragraph:

B5

-- A clone encoding a protein that specifically bound RANK was isolated and sequenced; the clone was referred to as 11H. An expression vector containing murine RANKL sequence, designated pDC406:muRANK-L (in *E. coli* DH10B), was

B5 deposited with the American Type Culture Collection, Manassas, VA (ATCC) on December 20, 1996, under terms of the Budapest Treaty, and given accession number 98284. The nucleotide sequence and predicted amino acid sequence of this clone are illustrated in SEQ ID NO:10. This clone did not contain an initiator methionine; additional, full-length clones were obtained from a 7B9 library (prepared substantially as described in US patent 5,599,905, issued February 4, 1997); the 5' region was found to be identical to that of human RANKL as shown in SEQ ID NO:13, amino acids 1 through 22, except for substitution of a Gly for a Thr at residue 9. --

At pages 30-31, please delete the paragraph at page 30, line 36 to page 31, line 10, which begins with "Addition of RANKL to DC..." and substitute therefor the following paragraph:

B6 -- Addition of RANKL to DC cultures significantly increased the degree of DC aggregation and cluster formation above control cultures, similar to the effects seen with CD40L. Sorted human CD1a<sup>+</sup> DC were cultured in a cytokine cocktail (GM-CSF, IL-4, TNF- $\alpha$  and FL), in cocktail plus CD40L (1 $\mu$ g/ml), in cocktail plus RANKL (1 $\mu$ g/ml), or in cocktail plus heat inactivated ( $\Delta$ H) RANKL (1 $\mu$ g/ml) in 24-well flat bottomed culture plates in 1 ml culture media for 48-72 hours and then photographed using an inversion microscope. An increase in DC aggregation and cluster formation above control cultures was not evident when heat inactivated RANKL was used, indicating that this effect was dependent on biologically active protein. However, initial phenotypic analysis of adhesion molecule expression indicated that RANKL-induced clustering was not due to increased levels of CD2, CD11a, CD54 or CD58. --

At page 31, please delete in its entirety the paragraph at lines 11-24, which begins with "The addition of RANKL..." and substitute therefor the following paragraph:

B7 -- The addition of RANKL to CD1a<sup>+</sup> DC enhanced their allo-stimulatory capacity in a mixed lymphocyte reaction (MLR) by at least 3- to 10-fold, comparable to CD40L-cultured DC (Figure 2). Allogeneic T cells (1x10<sup>5</sup>) were incubated with varying numbers of irradiated (2000 rad) DC cultured as indicated above in 96-well round bottomed culture plates in 0.2 ml culture medium for four days. The cultures were pulsed with 0.5 mCi [<sup>3</sup>H]-thymidine for eight hours and the cells harvested onto glass fiber sheets for counting on a gas phase  $\beta$  counter. The background counts for either T cells or DC cultured alone were <100 cpm. Values represent the mean  $\pm$  SD of triplicate cultures. Heat inactivated RANKL had no effect. DC allo-stimulatory